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MICROFLOCCULATION TESTS FOR SYPHILIS

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Based on "Syphilitic Tests for Syphilis"
of the U.S. Public Health Service

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STANDARD METHOD FOR PERFORMING THE MICROFLOCCULATION TEST FOR SYPHILIS

EQUIPMENT

1. Rotating Machine (Boerner-type shaker) to rotate 180 RPM.
2. Ring Maker, (The Fisher rapid ring maker may be used) which will prepare paraffin ring slides, similar to the Kline Slide.
3. Slide Holder to accommodate 2X3 inch slides.
4. Glass slides, flat bottom, 2x3 inches, with paraffin rings approximately 14mm in diameter. Glass slides with ceramic rings may also be used.
5. Bottles, glass stoppered or screw capped (tin foil lined), round 30 ml.
6. Syringes, Luer type, 1 or 2 ml. *
7. Hypodermic needles:
    a) 23 gauge needles, calibrated to deliver 100 drops of saline per ml. (Holding the syringe almost vertically will usually deliver the required amount.)
    b) 19 or 20 gage needles with the bevels cut off, calibrated to deliver 75 drops of antigen per ml when syringe is held vertically.
8. Pipettes - 0.2 ml. graduated in 0.01 ml. to deliver serum.
9. Microscope - 100 X magnification.

PREPARATION OF ANTIGEN

The antigen consists of:

Cholesterol - 0.9%

Cardiolipin - 0.03%

Lecithin - Quantity necessary to maintain reactivity of standard antigen.

* Syringes should be washed after use in water followed by an alcohol rinse, and then an ether rinse.

PREPARATION OF SERUM

Clear serum is heated in the 56°C water bath for 30 minutes. All serums containing visible particles after the heating period should be recentrifuged.

Serums tested more than 4 hours after the original heating period should be reheated for 10 minutes in the 56°C water bath.

THE SALINE SOLUTIONS

1. 0.9% saline solution is prepared by carefully weighting 900 mg. of previously oven dried sodium chloride, adding the dried NaCl to 100 ml. of distilled water. Filter the saline before use.

2. Buffer Saline - Formaldehyde, neutral, reagent grade, ml. 0.5
   Secondary sodium phosphate ($Na_2HPO_4+12H_2O$) gm. 0.093
   Primary potassium phosphate (KH_2PO_4), gm. 0.170
   Sodium chloride, A.C.S. gm 10.0
   Distilled water ml. 1000.0

The solution yields potentiometer readings of pH 6.0 + 0.1 and is stored in screw-capped or glass-stoppered bottles.

PREPARATION OF SLIDES

Paraffin rings are prepared (as for the Kline Test) by means of the Ring Maker (See Equipment).

All slides are cleaned as follows:

1) New Slides are cleaned with Bon Ami. The Bon Ami is allowed to dry and is then removed with a soft cloth.

2) Used slides are cleaned by first removing the paraffin with hot soapy water, then washed with soap and water and Bon Ami applied as described for New Slides.

   If slides are properly cleaned, serum will spread unaided when placed within the paraffin ring. If serum does not spread, do not use the slide, as it has not been sufficiently cleaned.

PREPARATION OF ANTIGEN EMULSION

1. Pippette 0.4 ml, of buffered saline to the bottom of a 30 ml. round, glass or screw-cap stoppered bottle.

2. All 0.5 ml. antigen (from the lower half of a 1.0 ml. pipette graduated to the tip) directly onto the saline while continuously but gently rotating the bottle on a flat surface.

Note: Antigen is added drop by drop but rapidly, so that approximately 6 seconds are allowed for each 0.5 ml. antigen. Pipette tip should remain in upper third of bottle and rotation should not be vigorous enough to splash saline onto pipette.

Preparation of Antigen Emulsion (Cont'd)

3. Blow last drop of antigen from pipette without touching pipette to saline.

4. Continue rotation of bottle 10 more seconds.

5. Add 4.1 ml. buffered saline from 5.0 ml. pipette.

6. Place top on bottle and shake vigorously for approximately 10 seconds.

7. Antigen emulsion is then ready for use and may be used during one day.

All antigen emulsions should be tested by using known positive and negative serum, as well as with saline. The particles of the antigen emulsion in the negative serum and the saline control must not appear too large. If the antigen particles are not of satisfactory size (as determined by experience) the emulsion should be discarded.

THE SERUM TEST

1. Pipette 0.04 ml., 0.2 ml. and 0.01 ml. heated serum to the 1st, 2nd and 3rd rings of the paraffin slides.

2. Add with needle and syringe, 2 and 3 drops of 0.9% saline to the 2nd and 3rd rings respectively (Each drop must contain 0.01 ml. This is accomplished by using the 23 gauge needle as explained in the section on "Equipment").

3. Add one drop of antigen emulsion to all three rings. (Each drop contains 1/75 ml. - using the 19 or 20 gauge needle, holding syringe vertically, as explained in the section on "Equipment").

4. Rotate the slide for four minutes at 180 R.P.M. on the Boerner-type rotator.

5. Read test immediately after rotation.

6. If clumps are present in the third ring, then dilute the serum 1:8 (1 part serum to 7 parts 0.9% saline) and repeat the test with the diluted serum.

The following chart summarizes the Serum Test:

PARAFFIN RING #	1	2	3		4	5	6
Serum ml.	0.04	0.02	0.01	Serum	.04	.02	0.01
Saline (Each drop 1/100 ml)	-	2	3	Diluted	-	2	3
Antigen emulsion (each drop 1/75 ml.)	1 drop	1 drop	1 drop	1:8	1 drop	1 drop	1 drop
Serum dilution	1:1	1:2	1:4		1:8	1:16	1:32

If clumps are present in ring 6 (1:32 dilution), dilute serum 1:64 and repeat test. A negative reaction must be obtained before test is concluded.

Reading and Reporting Test Results

1. Read tests microscopically at a magnification of 100 X

Aggregation of the antigen particles into large or small clumps is interpreted as degrees of reactivity.

READINGS	REPORT	
No clumping or very slight roughness - - - - - - -	Non Reactive	(N)
Small Clumps - - - - - - - - - - - - - - - - - - -	Weakly Reactive	(WR)
Medium and Large Clumps - - - - - - - - - - - - -	Reactive	(R)

2. Report results as follows:

Report results in terms of the greatest serum dilution that produces a Reactive result in accordance with the following examples:

Serum dilution:

1:1	1:2	1:4	1:8	REPORT
WR	N	N	N	Weakly Reactive undiluted or Weekly Reactive 1 dil.
R	WR	N	N	Reactive undiluted or Reactive 1 dil.
R	R	N	N	Reactive 1:2 or Reactive 2 dils.
N	N	N		Non Reactive